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論文題目	Transplantation of feeder-free human induced pluripotent stem cell-derived cortical neuron progenitors in adult male Wistar rats with focal brain ischemia （フィーダーフリー環境で誘導されたヒト多能性幹細胞由来大脳皮質神経細胞の成体雄ウィスターラット局所脳虚血モデルへの移植）		

（論文内容の要旨）

Transplantation of human neural stem cells (NSCs) has been reported to improved functional recovery in animal models of stroke and initiated clinical trials in limited number of patients. The discovery of human induced pluripotent stem cells (hiPSCs) represent a valuable treatment option for stroke cell therapy, as it avoids many ethical issues associated with the use of fetal or embryonic material, and generation of cortical neuron progenitors from hiPSCs potentially become a reliable source for stroke cell therapy. In this regards, there is no clinical-compliance protocol for the derivation of cortical neuron progenitor from hiPSCs. Another fundamental question in clinical-therapeutical perspectice, is how the stroke-induced microenvironment influences the survival, migration, neural differentiation and integration of the transplanted hiPSCs-derived cells.

To solve the issues, the feeder-condition for the derivation of cortical neurons was developed. The differentiation process was characterized by down-regulation of pluripotency genes (*POUF51* and *NANOG*) and up-regulation of neuronal genes (*PAX6, SOX1, FOXG1,* and *LHX2*). The cells were further characterized by immunocytochemistry of related cortical neuron markers (FOXG1, LHX2, CTIP2 and TBR1). The cells on days 25 were collected and transplanted into ipsilesional cerebral cortex of stroke animal models (n=10, 7 days after 90 minutes of proximal middle cerebral artery occlusion) and sham operated animals (n=10) for the control. In this study, all animals received daily cyclosporine A injection (10 mg/kg bw i.p.) for the immunosuppression. The animals were observed until 4 weeks post-transplantation and analyzed by immunohistochemistry. The transplanted cells were identified using anti-human nuclei antibody (RRID: AB_94090).

In this study, the transplanted cells in stroke animals extensively migrated toward the ischemic side compare to the sham animals, yet there was no difference in term of graft survival. All migrating cells were confirmed to be neuronal lineage ($Tuj1^{+}$/HNA^{+}), although reactive gliosis was observed both in ischemic border or surrounding the graft, there was no astrocytic differentiation from the cells ($GFAP^{+}$/HNA^{+}). The transplanted cells in the stroke exhibited a directed fiber-outgrowth (human NCAM) toward the ischemic side, while in sham animals there was no directional preference. Delayed-transplantation in stroke animals did not prevent ventricular enlargement (brain atrophy) nor promote endogenous neurogenesis. Despite of those, forelimb asymmetry (cylinder test) was better restored in transplanted animals compared with vehicle group (n=10). This effect might be related to the reduction of microglia infiltration in the ischemic border.

To this end, the results prove that the generation of transplantable hiPSC-derived cortical neuron progenitors could be obtained from the feeder-free condition.

（論文審査の結果の要旨）

ヒト人工多能性幹細胞（hiPSC）は脳梗塞に対する細胞移植治療の細胞源として期待されている。しかし、臨床応用に向けて、動物由来のフィーダー細胞や因子を用いない神経分化誘導が課題となっていた。また、梗塞脳における移植細胞の脳内での分化傾向について、正常脳と比較検討した報告はなかった。

本研究では、動物由来のフィーダー細胞や因子を除去するため、hiPSC（836B1 株）を合成ラミニン基質である Laminin-511 E8 上で動物因子非含有培地（Stemfit® AK03）を用いて培養した。大脳皮質神経前駆細胞は SFEBq 法（Dual SMAD + WNT inhibition）で作製し、分化開始 25 日目に免疫染色により神経分化を確認した。同日に細胞を回収し、中大脳動脈閉塞モデルラットの患側大脳皮質に移植した。4 週間後、免疫組織染色により移植細胞の生着や分化、神経突起伸展について評価を行った。

移植細胞の生存率や成熟神経細胞への分化について、対照群と梗塞群間で有意差は認められなかった。また、移植細胞のアストロサイトへの分化はみられなかった。一方、梗塞群では、虚血周辺脳への細胞の遊走および神経突起の伸展が有意に増加していた。

以上の研究は宿主環境が移植細胞の脳内動態に与える影響の解明に貢献し、hiPSC を用いた脳梗塞に対する細胞移植治療の実現に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成 31 年 2 月 19 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降